Docket No.: 2912960-001000 Application No.: 10/748,094

## Amendments to the Claims:

This listing of claims will replace all prior versions, and listings of claims in the application:

## **Listing of Claims:**

1. (Amended) A process for manufacture of long circulating non-pegylated liposomes comprising: dissolving one or more phospholipids and one or more sterols in a solvent or mixture of solvents;

hydrating the phospholipids and one or more sterols in a solvent or mixture of solvents; wherein the one or more phospholipids is a saturated phosphatidylcholine selected from the group consisting of distearoyl phosphatidylcholine (DSPC), hydrogenated soya phosphatidylcholine (HSPC) and mixtures thereof;

removing the solvent or mixture of solvents before of after hydrating the lipids and sterols and adding an aqueous hydration media to the phospholipids and sterols; or adding an aqueous hydration media to the phospholipids and sterols in the solution; and removing the solvent or mixture of solvents;

wherein the aqueous hydration media comprises ammonium sulfate and sucrose and; wherein the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present to form <u>long circulating</u> non-pegylated liposomes; and

wherein the aqueous hydration media comprises ammonium sulfate and sucrose;

sizing the non-pegylated liposomes formed to about 0.06 µm to form a liposomal eomposition; removing ammonium sulphate from extraliposomal hydration medium-salt from the liposomal composition by dialysis, ultrafiltration or column chromatography using a sucrose-histidine buffer solution;

and wherein the one or more phospholipids is a saturated phosphatidylcholine selected from the group consisting of distearoyl phosphatidylcholine (DSPC), dipalmitoyl phosphatidylcholine (DPPC), hydrogenated soya phosphatidylcholine (HSPC) and mixtures thereof; and wherein the one or more phospholipids exhibits a phase transition temperature of between 50 and 65°C; and

wherein the resulting non-pegylated liposomes have a blood circulation half life of at least 25 times longer than conventional non-liposomal formulations when tested in Swiss albino mice at equivalent doses.

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2. (original) The process of claim 1 wherein the amount of aqueous hydration media used is 30

ml for each mmole of phospholipid in the lipid solution.

3. (Currently amended) The process of manufacture of non-pegylated liposomes of claim 1

further comprising loading the liposomes with a therapeutic or diagnostic agent after removal of

the ammonium sulphate from the extraliposomal hydration medium.

4. (original) The process of claim 3, wherein the therapeutic agent is an antineoplastic agent.

5. (original) The process of claim 4, wherein the antineoplastic agent is selected from the group

consisting of Doxorubicin hydrochloride, Daunorubicin hydrochloride, and Epirubicin

hydrochloride.

6. (original) The process of claim 5, wherein the antineoplastic agent is Doxorubicin hydrochloride.

7. (original) The process of claim 1, wherein the molar ratio of phospholipid to sterol is from

about 1:0.1-1:2.

8. (previously presented) The process of claim 7, wherein the molar ratio of phospholipid to

sterol is about 1:0.7.

9. (previously canceled).

10. (previously presented) The process of claim 1, wherein the concentration of ammonium

sulfate in aqueous hydration media is not less than 125 mmoles/liter.

11. (previously canceled).

12. (previously presented) The process of claim 1, wherein the phospholipid has a minimum of

sixteen carbons fatty acid chain.

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13. (previously canceled).

14. (previously presented) The process of claim 1, wherein the phospholipid is distearoyl

phosphatidylcholine (DSPC) and wherein the sterol is cholesterol.

15. (original) The process of claim 1, wherein the non-pegylated liposomes are successively

extruded through series of filters having pore sizes from 0.4 µm to 0.05 µm for sizing.

16. (original) A liposome manufactured by the process of claim 1.

17. (original) The liposome of claim 16, wherein the phospholipid comprises distearoyl

phosphatidylcholine (DSPC) and the sterol comprises cholesterol.

18. (Original) The liposome of claim 16, wherein the non-pegylated liposome further comprises

a therapeutic or diagnostic agent.

19. (Original) The liposome of claim 18, wherein said therapeutic agent comprises an

antineoplastic agent.

20. (original) The liposome of claim 19, wherein the antineoplastic agent is selected from the

group consisting of Doxorubicin hydrochloride, Daunorubicin hydrochloride, and Epirubicin

hydrochloride.

21. (original) The liposome of claim 20, wherein the antineoplastic agent is Doxorubicin

hydrochloride.

22. (original) The liposome of claim 16, wherein the average size of liposome is 0.06 μm to 0.16

μm in diameter.

23-61. (previously canceled).

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62. (Currently canceled).

63. (New) A process for manufacture of non-pegylated liposomes comprising:

forming a lipid film by evaporating a solvent from a lipid solution comprising one or more phospholipids, a sterol, and a solvent; and

hydrating the lipid film by adding an aqueous hydration media to form a non-pegylated liposomal composition; wherein the aqueous hydration media comprises ammonium sulfate and sucrose and wherein the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present in the lipid solution; and

removing ammonium sulphate from extraliposomal hydration medium using a sucrosehistidine buffer solution.

64. (New) The process of Claim 63 wherein the aqueous hydration media comprises greater than 125 mM ammonium sulfate and 100 mM to 500mM sucrose.

65. (New) The process of Claim 63 wherein the aqueous hydration media comprises greater than 125 mM ammonium sulfate and 250 mM to 300 mM sucrose.

66. (New) The process of Claim 63 wherein the amount of histidine in the sucrose-histidine buffer is 1 mM to 100 mM.

67. (New) The process of Claim 63 wherein amount of histidine in the sucrose-histidine buffer is 8 to 12 mM.

68. (New) The process of Claim 63 wherein amount of histidine in the sucrose-histidine buffer is 10 mM.

69. (New) The proces of Claim 1 wherein the long circulating non-pegylated liposomes have a blood circulation half life of at least 25 times longer than conventional non-liposomal formulations when tested in Swiss albino mice at equivalent doses.